RESEARCH PAPER

Regional Variation in Retinal Ganglion Cell Thickness: Functional Correlation and Quadrant Specific Analysis in Glaucoma

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ABSTRACT

Purpose: The purpose of the study is to comprehend the variations in retinal ganglion cell thickness. (RGCs) in the different quadrants and hemispheres of the retina and the degree to which these differences correlate with aspects of visual function like glaucoma patients' contrast sensitivity (CS) and best corrected visual acuity (BCVA).

Materials and Methods: In the observational cross-sectional study design, 144 patients with glaucoma (out of 222 screened, with 78 exclusions) were enrolled at Shivom Eye Care, Delhi NCR, India over a period of 18 months using purposive sampling. Inclusion criteria consisted of patients with glaucoma, with intraocular pressure (IOP) of 22 mmHg or above, and a positive family history. Measurements of RGC thickness were performed by optical coherence tomography (Zeiss Cirrus HD 500) for the six retinal quadrants: superotemporal, superior, superonasal, inferonasal, inferior, and inferotemporal. Assessment of visual function involved BCVA as measured by a LogMAR chart and CS, as measured by the Pelli-Robson chart. Pearson correlation coefficients and independent t-tests were the statistical analyses performed.

Results: The average age of the research participants was 57.18±9.05 years, of which 50.7% were men, and the age range was 41 to 75. All of the participants had a positive family history. The mean IOP was measured to be 27.89±5.27 mmHg in the right eye and 28.09±5.39 mmHg in the left eye. The average RGC thickness showed minimal variation across the quadrants, with a mean thickness of 69.01±13.51 to 72.06±14.88 µm in the right eye and 68.58±16.00 to 73.47±14.35 µm in the left eye. The superior and inferior hemispheres showed no discernible changes (RE: $p=0.758$; LE: $p=0.537$). RGC thickness and BCVA were strongly negatively correlated in all quadrants (RE: $r=-0.694$ to -0.787, $p<0.0001$; LE: $r=-0.525$ to -0.623, $p<0.0001$). RGC thickness and CS had a significant correlation (RE: $r=0.680$ to 0.760, $p<0.0001$; LE: $r=0.539$ to 0.597, $p<0.0001$). RGC thickness and IOP were significantly correlated negatively (RE: $r=-0.764$, $p<0.0001$; LE: $r=-0.655$, $p<0.0001$).

Conclusions: RGC thickness is more evenly distributed across quadrants in glaucoma patients, with the superior quadrants having a greater mean thickness. There are strong correlations between RGC thickness and the respective visual performance parameters, indicating RGC thickness as clinically relevant biomarker to determine functional visual impairment due to glaucoma. This study further emphasizes the clinical value of RGC analysis in managing and tracking glaucoma in a quadrant-specific manner.

Keywords: Glaucoma; Retinal ganglion cell thickness; Optical coherence tomography; Visual acuity; Contrast sensitivity; Quadrant analysis; Structure-function correlation

How to cite this article: Sharma K, Prasar K, Pattnaik SS, Sarma B, Kalra N, Panda D. Regional Variation in Retinal Ganglion Cell Thickness: Functional Correlation and Quadrant Specific Analysis in Glaucoma. Int J Drug Deliv Technol. 2026;16(51s): 1618-1628. DOI: 10.25258/ijddt.16.51s.123

INTRODUCTION

Glaucoma is linked to gradual loss of retinal ganglion cells (RGCs) and loss of visual field, making it one of the leading causes of permanent blindness globally. Initially, the pathophysiology of glaucoma is complex and involves factors such as elevated intraocular pressure (IOP), vascular insufficiency, excitotoxicity, and neurodegeneration leading to RGC apoptosis and axonal degeneration. [1,2]. In order to manage glaucoma effectively and prevent vision loss, early detection and monitoring of changes to the structure of the retina is necessary.

By enabling the measurement of retinal layers, such as the retinal ganglion cell (RGC) layer and the retinal nerve fiber layer (RNFL), recent advancements in optical coherence tomography (OCT) have transformed the diagnosis and monitoring of glaucoma, accurately and non-invasively.[3,4] Although measuring RNFL thickness is a clinical standard and has been the subject of numerous studies, recent research indicates measuring RGC

thickness may further improve the diagnosis and prognosis of glaucoma.[5,6]

RGCs, have an asymmetric and inhomogeneous distribution in the retina, which is characterized by varying densities in the superior and inferior hemispheres and in different quadrants.[7,8] The pattern and course of glaucomatous damage may be influenced by these characteristics, which could illuminate why some areas of the retina are more affected in the early stages of glaucoma. Identifying regional differences in RGC thickness and their association with visual function is critical to establishing more effective diagnostic and monitoring strategies.

Many studies have documented the correlations between structural attributes (parameters of the optic disc and RNFL thickness) and functional attributes (contrast sensitivity and visual field sensitivity) in the structure-function relationship in glaucoma.[9,10] Nonetheless, the relationship between the thickness of RGCs in various retinal quadrants and the multifactorial parameters of visual function is still incompletely defined. Understanding such parameters, or the lack thereof, may contribute to the elucidation of the phenomena of vision loss in glaucoma and the associated clinical features.

Testing of Contrast Sensitivity (CS) is now viewed as a significant functional evaluation of glaucoma and, in many cases, it is the first to identify glaucoma related deficits as it is more sensitive than standard visual acuity testing. [11,12] CS testing evaluates vision quality at various spatial frequencies and, in contrast to high-contrast visual acuity testing, it measures a wider range of vision function to simulate better everyday vision. There is a lack of detailed exploration into the relationship of RGC thickness and CS in various regions of the retina.

The Indian populace possesses distinctive epidemiological features pertaining to glaucoma, including a high incidence of primary angle-closure glaucoma, and unique genetic risks.[13,14] In addition, the obstacle of late presentation and restricted availability of advanced clinical services in various areas of the country underscores the requirement for easily measurable and objectively applicable parameters for the evaluation of glaucoma. The study of patterns of RGC thickness in glaucoma patients from India may provide evidence of population-specific characteristics useful for refinement of screening and management approaches.

Objectives and Reasoning Behind the Study

Quite a bit has been studied regarding the thickness of the RNFL and glaucoma. However, in the Indian population, there does not seem to be a study exploring the various thicknesses of RGC quadrants and their corresponding associations with visual function parameters and with a clearly defined cohort of glaucoma's. This study aims to provide a clear analysis of the RGC sub-type thicknesses and the functions that go along with them in this group.Therefore, this study aims to achieve the following:

1. Measure the thickness of RGCs in the six aforementioned quadrants in the glaucoma population using spectral-domain OCT.
2. Determine the thickness discrepancies of RGCs in the superior vs. inferior hemispheres.
3. Determine any correlational relationships of quadrant RGC thickness with visual acuity.
4. Determine the relationships of RGC thickness with contrast sensitivity in different regions of the retina.
5. Determine any correlational relationships of RGC thickness with intraocular pressure.
6. Determine the relationships of RGC thickness with the demographic attributes of the population.

We predict that there will be variations in the thickness of RGCs and this thickness will correlate with the parameters of visual function. These relationships will be important gaps that can be filled in order to have further a clinical perspective on the evaluation and follow-up of glaucoma's.

METHODS AND MATERIALS

2.1 Study Design

This observational cross-sectional study was conducted at Shivom Eye Care, a tertiary eye care facility in Delhi National Capital Region (NCR), India. The Institutional Ethics Committee approved the research protocol prior to patient recruitment. The study time frame was 18 months, January 2023 to June 2024.

2.2 Sample Size and Sampling Technique

Initially, 222 patients were screened for eligibility to participate in the study. After applying inclusion and exclusion criteria, 78 patients were excluded regarding the study, and 144 were included. The final study population consisted of 144 glaucoma patients who had undertaken all the study procedures. Purposive sampling was utilized to achieve the study inclusion of patients with specific glaucoma diagnoses, who had documented elevated IOP, and a familial history of the condition.

2.5 Inclusion Criteria

In the study, participants were included if they met the criteria set out. Such subject was over the age of 18, had been diagnosed with glaucoma by an eye doctor, and had undergone the necessary steps of gonioscopy, fundoscopic exam, and visual fields. Inclusion criteria also included an IOP of 22 mmHg or greater, one or both eyes, measured by Goldmann applanation tonometry. Individuals with glaucoma in the family were clinically assessed to be positive for primary open-angle glaucoma in one first-degree relative. Further, study participants needed to possess sufficient cognitive capacity to comprehend the study and complete the informed consent documents as well as pass OCT testing.

2.6 Exclusion Criteria

If a patient had a condition that may impact the study's evaluation and results, they were all omitted. This covers situations when motion artifacts and corneal opacity or other media opacities result in low-quality OCT pictures. Retinal vascular occlusions, diabetic retinopathy, and other retinal disorders were also excluded from the research. Patients with the optic nerve and visual pathway disorders not caused by glaucoma were also excluded. These included patients with demyelinating diseases or disorders from intracranial pathologies. Patients with a spherical equivalent refractive error of more than ±5.00 diopters were also excluded. Patients with ocular hypertension without the presence of glaucomatous optic neuropathy or visual field defects were excluded as were those who had intraocular surgery in the previous 6 months, and patients who were not motivated or able to complete study tasks.

2.5 Study Procedures and Instrumentation

2.5.1 Visual Acuity Assessment

Using the Log MAR charts were evaluated for both best corrected visual acuity (BCVA) and uncorrected visual acuity (UCVA). This was done from a distance of 4 meters and under standard lighting of 85 cd/m². Each assessment was done monocularly with the fellow eye covered.

BCVA was assessed after the manifest refraction and trial frame adjustments. A LogMAR value was documented for the smallest line that was read with 3 or more letters correct.

2.5.2 Measurement of Contrast Sensitivity

Using the Pelli-Robson chart, contrast sensitivity was measured. This chart evaluates the contrast threshold at lower spatial frequency (1 cycle per degree). It was placed 1 meter away with 60-120 cd/m² of illumination. Each eye was tested monocularly and both eyes were tested together. The patients were instructed to read the letters from left to right. and to go down the chart until they could not tell the orientation of the letters. The score for contrast sensitivity was obtained from the log contrast sensitivity of the last set of 3 letters, with at least 2 correct.

2.5.3 Measurement of Intraocular Pressure

Goldmann applanation tonometry was used to measure IOP. This is the standard technique for professional IOP measuring. To reduce the impact of diurnal fluctuation, measurements were made between 10 AM and 12 PM. Sodium fluorescein and 0.5% proparacaine hydrochloride, a corneal numbing agent, were utilized. Each eye was measured three times, and the average of the three readings was calculated. For every measurement session, the tonometer was calibrated.

2.5.4 Slit Lamp Biomicroscopy.

A slit lamp examination was conducted to observe the anterior segment structures and detect any possible pathologies that would disqualify the participants or affect quality of the images taken. This appraisal covered the assessment of the cornea, anterior chamber, iris, lens, and pupil.

2.5.5 Fundus Examination

A dilated fundus examination was performed using 90D non-contact lens and slit lamp biomicroscopy after pupil dilation was obtained using 1% tropicamide and 2.5% phenylephrine HCL. On the optic disc, the examination included values of the cup-to-disc ratio, characteristics of the neuroretinal rim, location of any disc hemorrhages, and presence of peripapillary atrophy. The examination was done to end the presence of any concomitant disease of the retina, which included assessment of the macula and peripheral retina.

2.5.6 Optical Coherence Tomography Imaging

The Cirrus HD-OCT 500 by Zeiss (Carl Zeiss Meditec, Dublin, CA, USA), a spectral OCT system with an axial resolution of 5 µm and a transverse resolution of 15 µm, was used for retinal imaging. To retrieve volumetric data from the specified area of the macula, the macular cube protocol (512 × 128 scan pattern) was utilized. Software for ganglion cell analysis preemptively divided the plexiform layer of the ganglion cell-inner and the cell-complex and provided thickness maps.

Measurements for RGC thickness were conducted for six quadrants of the retina surrounding the fovea, using a 14.13 mm² elliptical annulus (0.5 mm inner radius, 2.0 mm outer radius). The six quadrants were: ST (Superotemporal): superior-temporal quadrant, S (Superior): superior quadrant, SN (Superonasal): superior-nasal quadrant, IN (Inferonasal): inferior-nasal quadrant, I (Inferior): inferior quadrant, IT (Inferotemporal): inferior-temporal quadrant.

Each quadrant had its average and minimum RGC thickness recorded. The thickness for the superior hemisphere was obtained by averaging the thickness of the ST, S, and SN quadrants, while the inferior hemisphere thickness was obtained by averaging the IN, I, and IT quadrants. Only scans with a signal strength equal to or greater than 7/10 were analysed, while scans with artifacts, segmentation errors, and poor centration were excluded, necessitating repeat scans.

2.6 Statistical Analysis

The statistical analysis was done using the SPSS software. Continuous variables were calculated using the mean and standard deviation, while categorical variables were calculated using frequency and percentage. Data distribution was assessed for normality and similarity using the Shapiro-Wilk test and Q-Q plots.

The associations between the RGC thickness measurements and the continuous visual function parameters (BCVA, CS, and IOP) were evaluated using Pearson correlation coefficients. The following criteria were used to interpret the correlation: r=0.00-0.19 (very weak), 0.20-0.39 (weak), 0.40-0.59 (moderate), 0.60-0.79 (strong), and 0.80-1.00 (very strong).

Mean RGC Thickness was compared using independent sample t-tests between the superior and inferior hemispheres and male and female subjects. The difference between the age groups was assessed using Analysis of Variance (ANOVA).

All statistical tests were done in both directions and p values below 0.05 were considered significant. The explorative nature of some analyses means no adjustments for multiple comparisons were made. The calculation of sample size was made to show that 144 patients would give over 80% power to detect moderate correlations (r greater than 0.25) at the 0.05 significance level.

2.8 Ethical Considerations

Informed consent was obtained from all participants after they were provided details about the study procedures, as well as any risks and benefits. Patients were made aware that they could leave the study at any time without any repercussions to their clinical care. Participants were not charged for any of the study activities. Confidentiality was upheld by keeping patient information anonymous and securely storing study documents.

RESULTS

3.1 Study Population and Demographics

During the study's 16-month, 222 patients were screened for eligibility. Of the screened patients, 144 were enrolled after applying the inclusion and exclusion criteria and completing all study assessments for a final study retention of 64.9%. The most common exclusion criteria for patients were of concurrent retinal disease (n=28, 35.9%), corneal opacities that impaired imaging (n=19, 24.4%), refractive errors beyond study criteria (n=15, 19.2%), ocular hypertension lacking glaucomatous damage (n=11,

14.1%), and for 5 patients (6.4%), refusal or an inability to complete study assessments.

The mean age of participants enrolled in this trial was 57.18 ± 9.05 years (41-75 years). Of the participants 42 (29.2%) were 41-50 years of age, 45 (31.3%) were 51-60 years of age, 49 (34.0%) were 61-70 years of age, and 8 (5.6%) were 71-75 years of age. There were 73 (50.7%) male and 71 (49.3%) female participants, indicating equal gender distribution. 100% of participants met the inclusion criteria as all reported a positive family history of glaucoma in first degree relatives.

Table 1: Characteristics of Participants (N=144)

Parameter	Value	Percentage/Range
Total Patients	144	64.9% of screened
Age (years)	57.18 ± 9.05	41-75
Male	73	50.7%
Female	71	49.3%
Family History	144	100%

3.2 IOP and Refraction Status

The IOP readings correspond to values that would confirm a diagnosis of glaucoma. Right eye IOP was (mean ± SD) 27.89 ± 5.27 mmHg (range: 22 - 45 mmHg) and left eye IOP was 28.09 ± 5.39 mmHg (range: 22 - 46 mmHg). Right and left eye IOP values were not significantly different (p=0.745). All participants IOP 22 mmHg in at least one eye.

All participants had their refractive corrections done within the limits of the study. Right eye and left eye mean spherical equivalent refractive error were - 1.24 ± 2.18 and -1.31 ± 2.15 diopter respectively and were all within the inclusion criterion of ± 5.00 diopters.

3.3 Visual Function Parameters

3.3.1 Visual Acuity

The right eye's UCVA was 0.389 ± 0.285 LogMAR, whereas the left eye was 0.398 ± 0.291 LogMAR. Best corrected visual acuity (BCVA) rose to 0.247 ± 0.220 LogMAR in the right eye and 0.253 ± 0.231 LogMAR in the left eye following refractive correction. Refractive correction improved visual acuity by 0.142 LogMAR units in the right eye and 0.145 LogMAR units in the left. For both eyes, the improvement was statistically significant ($p<0.001$). The BCVA values also have a good amount of intra-participant variability ranging from 0.00 LogMAR (20/20 Snellen equivalent) to 0.90 LogMAR (about 20/160), which illustrates the variability of visual impairment due to glaucoma in the study cohort.

3.3.2 Contrast Sensitivity

The study population exhibited decreased contrast sensitivity as evidenced by testing conducted using the Pelli-Robson contrast sensitivity chart. The right eye averaged a contrast sensitivity of 1.272 ± 0.324 log units (range: 0.30-1.95), and the left eye averaged 1.229 ± 0.334 log units (range: 0.45-1.80). Binocular contrast sensitivity had best performance at 1.397 ± 0.263 log units (range: 0.75-1.95), indicating the effects of binocular summation. In comparison to the monocular measurements, the binocular contrast sensitivity values reflected a statistically significant increase ($p<0.001$), with a mean binocular advantage of 0.125 log units versus contrast sensitivity of the right eye and 0.168 log units versus contrast sensitivity of the left eye.

Table 2: Parameters on Visual Function and Intraocular Pressure

Visual Parameter	Right Eye	Left Eye
IOP (mmHg)	27.89 ± 5.27	28.09 ± 5.39
UCVA (LogMAR)	0.389 ± 0.285	0.398 ± 0.291
BCVA (LogMAR)	0.247 ± 0.220	0.253 ± 0.231
CS (log units)	1.272 ± 0.324	1.229 ± 0.334

IOP stands for intraocular pressure; UCVA for uncorrected visual acuity; BCVA for best corrected visual acuity; and CS for contrast sensitivity.

3.4 Retinal Ganglion Cell Thickness: Quadrant Specific

3.4.1 Distribution of RGC Thickness in Right Eye (OD)

From the Spectral Domain OCT analysis of the right eye RGC Thickness, the allocation across the six quadrants seems to be evenly spread out. The superonasal quadrant has the most mean thickness of 72.06 ± 14.88 µm (with 22-98 µm variance) and has competitive thickness with the inferotemporal quadrant of 70.84 ± 13.90 µm (with 23-105 µm variance). The inferior quadrant has the least average RGC thickness of 69.01 ± 13.51 µm (with 25-93 µm variance). The other quadrants have thickness averages of: superotemporal 69.69 ± 14.46 µm, superior 69.58 ± 14.82 µm, and inferonasal 70.03 ± 13.95 µm. In summary, the mean RGC thickness across the right eye quadrants may be classified to be 70.00 ± 13.13 µm.

The results of the analysis of variance (ANOVA) on the mean RGC thickness for the six quadrants of the right eye shows no significant difference at ($F= 1.42$, $p= 0.214$), implying that, in spite of the glaucoma damage, RGC cells are homogeneously distributed in most quadrants. The however, Coefficient of Variation ranged from 19.6% to 21.3% which suggests lesser average thickness of RGC across the quadrants.

Table 3: Distribution of Quadrant-Specific Retinal Ganglion Cell Thickness in Right Eye (N=144)

Quadrant	Mean ± SD (µm)	Range (µm)	CV (%)
Superotemporal	69.69 ± 14.46	16-110	20.7
Superior	69.58 ± 14.82	23-96	21.3
Superonasal	72.06 ± 14.88	22-98	20.6
Inferonasal	70.03 ± 13.95	27-98	19.9
Inferior	69.01 ± 13.51	25-93	19.6
Inferotemporal	70.84 ± 13.90	23-105	19.6
Average	70.00 ± 13.13	26-97	18.8

SD: Standard Deviation; CV: Coefficient of Variation.

3.4.2 Distribution of Retinal Ganglion Cell (RGC) Thickness in the Left Eye (OS)

Similar to the right eye, the analysis of RGC thickness in the left eye showed fairly uniform distribution across all quadrants. While the maximum average thickness was noted in the superotemporal quadrant at 73.47 ± 14.35 µm (with a range from 26 to 105 µm), the average thickness of the superonasal quadrant was the lowest at 68.58 ± 16.00 µm (with a range from 9 to 98 µm). The superonasal quadrant contained one extremely low value of 9 µm, which indicates the presence of severely localized RGC loss in one patient suffering from advanced glaucoma. The average measurements for the remaining quadrants are: superior 71.39 ± 14.84 µm, inferonasal 69.83 ± 14.94 µm, inferior 69.42 ± 15.41 µm, and inferotemporal 71.13 ± 15.71 µm. The mean RGC thickness across all quadrants of the left eye was 70.86 ± 13.59 µm.

Right and left eye overall average RGC thickness showed no statistically significant difference (70.00 ± 13.13 µm vs. 70.86 ± 13.59 µm, p=0.563), which points to symmetrical involvement of RGC in bilateral glaucoma. On the contrary, a paired t-test of quadrants between the two eyes revealed superotemporal (p=0.028) and superonasal (p=0.048) quadrants as having significant difference, suggesting asymmetric involvement of the superior region in some patients.

Table 4: Quadrant-Specific RGC Thickness in Left Eye (N=144)

Quadrant	Mean ± SD (µm)	Range (µm)	CV (%)
Superotemporal	73.47 ± 14.35	26-105	19.5
Superior	71.39 ± 14.84	21-97	20.8
Superonasal	68.58 ± 16.00	9-98	23.3
Inferonasal	69.83 ± 14.94	19-98	21.4
Inferior	69.42 ± 15.41	15-103	22.2
Inferotemporal	71.13 ± 15.71	25-100	22.1
Average	70.86 ± 13.59	28-98	19.2

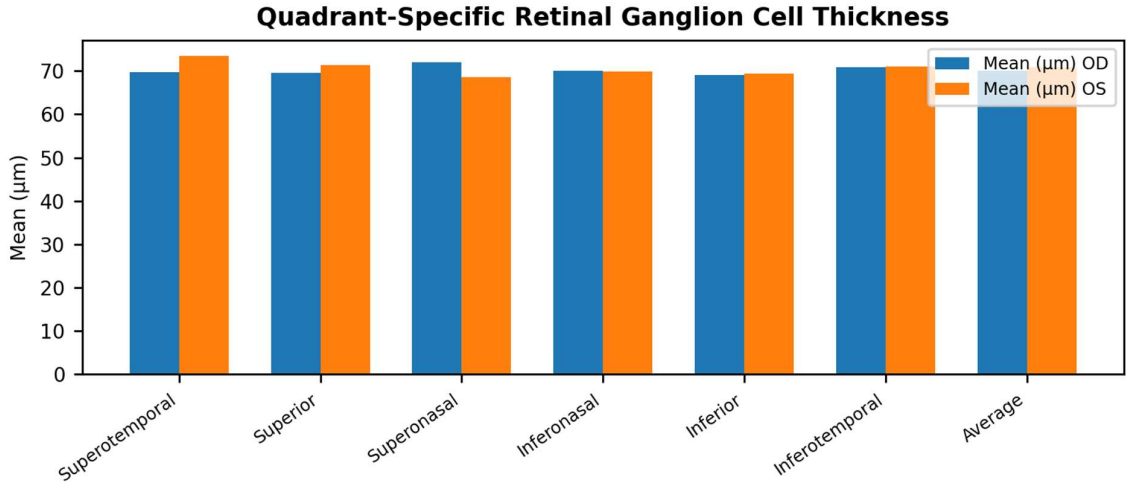

Fig 1: Distribution of Quadrant-Specific Retinal Ganglion Cell Thickness in OD & OS (N=144)

3.5 Comparison Between Superior and Inferior Hemispheres

To analyze RGC thickness in the hemispheres, the means from the superotemporal, the superior, and the superonasal was considered as the superior regions and the inferonasal, the inferior, and the inferotemporal as the inferior regions. The thickness of RGCs in the superior hemisphere of the right eye was, on average, 70.45 ± 13.84 µm and in the inferior hemisphere, 69.96 ± 12.76 µm. The difference of the two hemispheres, 0.49 µm, was found to be

insignificant (t=0.308, p=0.758) meaning that RGCs were equally present in both hemispheres.

Also, in the left eye, the RGC thickness in the superior hemisphere (71.15 ± 13.53 µm) was slightly higher than that in the inferior hemisphere (70.13 ± 14.43 µm) by 1.02 µm; however, this difference was also statistically insignificant (t=0.618, p=0.537). This means that there was almost the same amount of RGC loss due to glaucoma in both the inferior and superior regions of the retina, which is in contrast to the common belief in the field that early glaucoma preferentially affects the inferior field.

Table 5: Comparing Superior and Inferior Hemisphere RCG Thickness

Parameter	Right Eye (OD)	Left Eye (OS)
Superior Hemisphere (µm)	70.45 ± 13.84	71.15 ± 13.53
Inferior Hemisphere (µm)	69.96 ± 12.76	70.13 ± 14.43
Difference (µm)	0.49	1.02
p-value	0.758	0.537

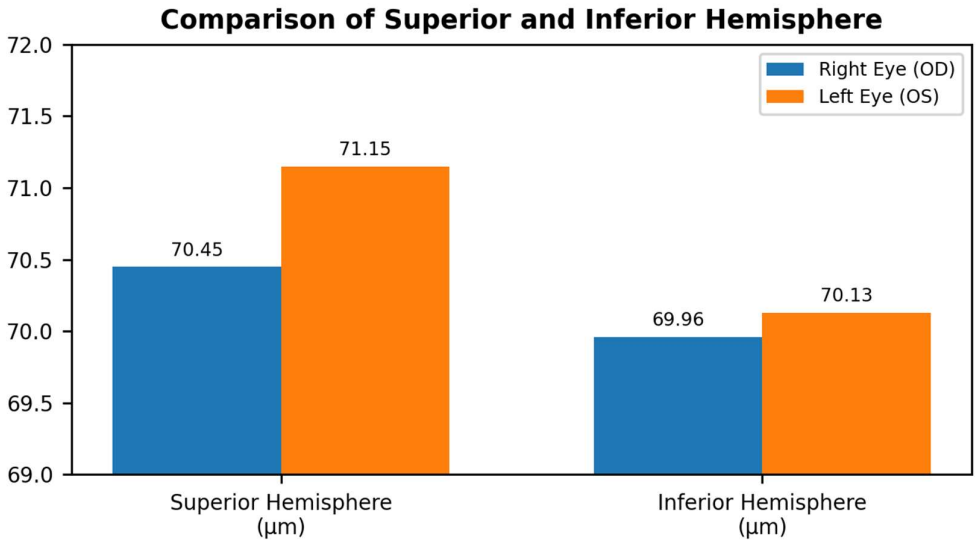

Fig 2: Comparing Superior and Inferior Hemisphere RCG Thickness

3.6 Structure-Function Correlations: RGC Thickness and Visual Acuity

3.6.1 Correlations in Right Eye

A Pearson correlation assessment indicated that in every RGC right eye quadrant there is a correlation RGC thickness and BCVA. The most significant correlation in the superior quadrant was documented (r=-0.787, p<0.0001), then in the superior temporal and inferior quadrants there were superotemporal (r=-0.771, p<0.0001) and inferior (r=-0.761, p<0.0001) quadrants. The greater the interquartile range, the weaker correlation; however, the the superonasal and inferonasal quadrants are of similar weakness (r=-0.694 and r=-0.711), but both p<0.0001 and are of significance. The inferotemporal quadrant correlation is r=-0.718 (p<0.0001). The greatest correlation coefficient was reported to be in the average RGC thickness, demonstrating a vast correlation with the BCVA (r=-0.789, p<0.0001).

3.6.2 Correlations on Left Eye

As far as correlation of left eye with average RGC thickness and BCVA is concerned, the correlation was strong but weaker than the right eye's superior: inferonasal quadrant has the strongest correlation coefficient (r=-0.623, p<0.0001), followed by superotemporal (r=-0.602, p<0.0001). superior and inferior temporal components are equal (r=-0.592 and r=-0.561 respectively, `p<0.0001). The correlation coefficient in inferior and superonasal quadrants is of minor significance (r=-0.556 and r=-0.525 respectively, p<0.0001). In average left RGC the coefficient is strong with BCVA, (r=-0.659, p<0.0001).

Table 6: Correlation between RGC Thickness and BCVA by Quadrant

Quadrant	Right Eye r (p)	Left Eye r (p)
Superotemporal	-0.771 (<0.0001)	-0.602 (<0.0001)
Superior	-0.787 (<0.0001)	-0.592 (<0.0001)
Superonasal	-0.711 (<0.0001)	-0.525 (<0.0001)
Inferonasal	-0.694 (<0.0001)	-0.623 (<0.0001)
Inferior	-0.761 (<0.0001)	-0.556 (<0.0001)
Inferotemporal	-0.718 (<0.0001)	-0.561 (<0.0001)
Average	-0.789 (<0.0001)	-0.659 (<0.0001)

Negative correlation indicates that thinner RGC corresponds to poorer (higher LogMAR) visual acuity.

3.7 Structure-Function Correlations: RGC Thickness and Contrast Sensitivity

3.7.1 Right Eye Correlations

Every right eye quadrant exhibited strong positive correlations between RGC thickness and contrast sensitivity. Average RGC thickness had the greatest correlation with contrast sensitivity ($r=0.769$, $p<0.0001$). For the individual quadrants, the superior region had the greatest correlation ($r=0.760$, $p<0.0001$) followed by superotemporal, with a correlation of $r=0.741$ ($p<0.0001$), and inferior, $r=0.740$ ($p<0.0001$). Inferotemporal, superonasal, and inferonasal quadrants had slightly lower correlations of $r=0.716$, 0.702, and 0.680, respectively (all $p<0.0001$) but the correlations are still considered strong. The positive correlations indicate that increased RGC thickness was linked to improved contrast sensitivity.

3.7.2 Left Eye Correlations

The left eye demonstrated relationships of moderate to strong positive RGC thickness to contrast sensitivity for all the quadrants, albeit weaker than the right eye. Average left eye RGC thickness and CS yielded correlation of 0.640 and $p<0.0001$. The highest in the inferonasal quadrant showed 0.597 ($p<0.0001$) followed by the superior quadrant with 0.577 ($p<0.0001$). Inferior, superotemporal, inferotemporal, and superonasal quadrants lower correlations of 0.562, 0.548, 0.557, and 0.539 respectively (all $p<0.0001$). These positive correlations for all quadrants describe the clinical significance of RGC thickness as a structural correlate of contrast sensitivity function.

Table 7: The Relationship of Quadrant-Based RGC Thickness with Contrast Sensitivity

Quadrant	Right Eye r (p)	Left Eye r (p)
Superotemporal	+0.741 (<0.0001)	+0.548 (<0.0001)
Superior	+0.760 (<0.0001)	+0.577 (<0.0001)
Superonasal	+0.702 (<0.0001)	+0.539 (<0.0001)
Inferonasal	+0.680 (<0.0001)	+0.597 (<0.0001)
Inferior	+0.740 (<0.0001)	+0.562 (<0.0001)
Inferotemporal	+0.716 (<0.0001)	+0.557 (<0.0001)
Average	+0.769 (<0.0001)	+0.640 (<0.0001)

Positive correlation means that thicker RGCs are associated with better contrast sensitivity.

3.8 RGC Thickness and Intraocular Pressure

The examination of RGC thickness and IOP revealed significant negative correlations for both eyes. In the right eye, average RGC thickness had a strong negative correlation with IOP ($r=-0.764$, $p<0.0001$), meaning that thinner RGC layers were associated with higher IOP measurements. The left eye showed a similar but somewhat less pronounced negative correlation ($r=-0.655$, $p<0.0001$) than the right. This is consistent with the proposed pathophysiological mechanisms wherein high IOP results in the structural and functional loss of RGCs in glaucoma. The relatively higher negative correlation measured in the right eye could possibly indicate more measurement variance or could reflect more advanced and asymmetric disease progression in some of the subjects.

3.9 Age-Related Variations in RGC Thickness

Age group analysis showed trends indicating decreased RGC thickness with age in both eyes. In right eye, mean RGC thickness decreased from 73.45 ± 12.89 µm in 41-50 years age group to 67.25 ± 9.35 µm in 71-75 years age group, resulting in a decrease of 6.20 um (8.4%) over the entire age spectrum. Nevertheless, the Pearson correlation of age and RGC thickness in the right eye reflect a very weak inverse correlation, which is close to, but not statistically significant ($r=-0.159$, $p=0.056$).

In left eye, age-related RGC thinning was more significant and the decrease was statistically significant. Mean RGC thickness decreased from 75.31 ± 15.50 µm in the youngest age group to 66.50 ± 6.80 µm in the oldest age group, which is an 8.81 µm (11.7%) decrease. The correlation of age and RGC thickness in the left eye was moderate and statistically significant ($r=-0.254$, $p=0.002$) indicating that RGC loss, in part, due to age, occurs independent of glaucomatous damage.

Table 8: RGC Thickness Analysis Based on Age

Age Group	n	OD RGC (µm)	OS RGC (µm)
41-50 years	42	73.45 ± 12.89	75.31 ± 15.50
51-60 years	45	69.40 ± 16.52	70.80 ± 14.94
61-70 years	49	68.04 ± 9.64	67.82 ± 10.16
71-75 years	8	67.25 ± 9.35	66.50 ± 6.80
Correlation (r, p)		-0.159, 0.056	-0.254, 0.002

3.10 Comparison of RGC Thickness Based on Gender

Analysis of the data based on gender provided interesting findings, especially on right eye RGC thickness. Male patients (n = 73) showed RGC thickness of 67.75 ± 14.48 µm. On the other hand, female patients (n = 71) showed RGC thickness of 72.30 ± 11.30 µm, which is significantly higher than that of the male patients ($t = -2.087$, $p = 0.039$). Thus, the difference of 4.55 µm translates to 6.7% higher RGC thickness in females than in males for the right eye.

On the contrary, there was no difference in left eye RGC thickness based on gender, where males showed 71.27 ± 12.78 μm and females 70.33 ± 14.52 μm ($t = 0.414$, $p = 0.680$). This paradox on the differences between the right and left eye may probably be due to chance. However, other unexplained factors may be at play, thus producing the shown findings on right eye RGC thickness, which may be related to the gender of the patients. These findings may also suggest that there are differences in the patterns of glaucomatous progression and/or RGC loss that are dependent on gender.

DISCUSSION

Insights into the distribution of regional retinal ganglion cell (RGC) thickness with functional correlates are provided for the first time in detail in this study that involved 144 glaucoma patients. Patients showed uniformity of retinal RGC thickness in all four retinal quadrants in spite of the existence of glaucomatous damage. There are strong structure-function relationships with visual acuity and contrast sensitivity and these relationships were shown to intraocular pressure. The findings could assist in understanding the pathophysiology of glaucoma and the evaluation of clinical assessment frameworks.

4.1 RGC Thickness Disparity

The analysis of the study showed a uniformity of RGC thickness in all retinal quadrants, with the right eye showing thickness in the range of 69.01 to 72.06 μm and the left eye with thickness in the range of 68.58 to 73.47 μm. This somewhat contradicts findings of studies of healthy eyes showing a peak RGC density, particularly at a parafovea. The apparent homogeneity of the glaucoma cohort is more likely to reflect diffuse RGC loss and not a failure to retain the anatomical distribution pattern.

The greater thickness in the superior quadrants, particularly the right eye superonasal and the left eye superotemporal, can potentially be explained by a few reasons. First, there is normal anatomy that demonstrates the presence of a greater RGC density in the superior regions and less in the inferior in normal healthy eyes. [17] second, the typical and common patterns of glaucomatous damage usually have a greater involvement of the inferior retina, which corresponds to defects in the visual fields that are superior. that could spare some of the superior RGC populations in some of the patients.[18] However, the fact that there is a small magnitude of these differences and that these differences are not significant in the superior and inferior differences indicate that the patients in the cohort had diffuse and advanced glaucoma damage in all regions of the retina.

With a comparison of the superior and inferior left and right hemispheres, there is no significant differences in either of the eyes, being $p=0.758$ in the right and $p=0.537$ in the left, which goes against the ideas that describe inferior retinal and superior field vulnerability in the early stages of glaucoma[19]. the case in hand can be based on the characteristics of the patients that were in the study as they all had IOP that were greater than or equal to 22 mmHg and most of the patients had advanced disease with visual impairments. Most of the early studies that support inferior damage focused on early glaucoma or the pre-perimetric stages. In the current study, the cohort had advanced stages and in the disease process, the damage involved both superior and inferior parts of the retina.

4.2 Structure-Function Correlations: Visual Acuity

One of the most significant observations of this study is the strong negative correlation of RGC thickness and BCVA across all quadrants (right eye $r = -0.694$ to -0.787, left eye $r = -0.525$ to -0.623, all $p < 0.0001$). These values are stronger than most studies investigating relationships between RNFL thickness and visual acuity, meaning that measuring RGC thickness may offer a better functional correlation than RNFL assessments.[20,21]

The stronger correlations for the right eye as compared to the left eye across all quadrants is an interesting observation. Three factors may explain this. First, the left eye may have poorer quality imaging due to variability in the imaging on one side versus the other, or poorer quality images. Second, in a number of patients, the right eye may have been the dominant eye and as such, the patient may have been less aggressive in fixating to the right during OCT imaging, thus, obtaining better quality functional metrics. Third, one eye may have been more severely affected by glaucoma than the other, leading to asymmetry in disease progression and thus, different levels of structure-function correlation.

Average RGC thickness having the largest correlation with BCVA (right eye $r=-0.789$, left eye $r=-0.659$) means global RGC assessment has predictive value for function. Nevertheless, individual quadrant analysis showing strong correlations suggests that, in addition to global measures, average RGC thickness values can provide predictive value and may reveal localized damage that is not captured in global measurements. This demonstrates the value in clinical practice of detailed quadrant-specific RGC analysis over global average thickness assessments.

4.3 Structure-Function Correlations: Contrast Sensitivity

The prominent positive correlations regarding RGC thickness and contrast sensitivity (right eye $r=0.680$ to 0.769; left eye $r=0.539$ to 0.640; all $p<0.0001$) further establishes RGC thickness as a potentially valid clinically relevant biomarker. Contrast sensitivity measures the visual processing capabilities spanning beyond the high-contrast visual acuity which may offer the opportunity to identify functional deficits more restoratively and reflect the day-to-day visual experience more accurately than other measures.[22,23]

The strength of our correlations is comparable or exceeds the scant literature regarding RNFL thickness and contrast sensitivity within the context of glaucoma.[24,25] The notable strong correlation of average right eye RGC thickness with contrast sensitivity ($r=0.769$) implies RGC evaluation as a structural correlate of value for consideration for this important functional component. The individual quadrants displayed correlations which, although still strong, were slightly less than this average value; this suggests that contrast sensitivity extends from a combination of global and regional measures of RGC cross-section area.

The robust RGC-contrast sensitivity correlations and their biological plausibility stems from the RGCs' own functional properties. Various RGC subtypes exhibit their own unique spatiotemporal processing, and the magnocellular system is specifically crucial for low spatial and contrast sensitivity.[26] There may also be glaucomatous

RGC loss impacting multiple cell types, although there has been a particular proposed selective susceptibility of magnocellular neurons which may be crucial for contrast sensitivity.[27]

4.4 RGC Thickness and Intraocular Pressure

The significant inverse correlations of RGC thickness and IOP (right eye r=-0.764, left eye r=-0.655, both p<0.0001) support the role of IOP in glaucomatous RGC degeneration. The strength of these correlations imply that in the study population, all of whom had IOP ≥22 mmHg, elevated IOP is a primary factor responsible for RGC loss. This is consistent with the volume of evidence that recognizes elevated IOP as the most significant risk factor for the worsening of glaucoma, and highlights the role of IOP lowering in decreasing the rate of glaucoma progression. [28,29]

Nonetheless, the moderate strength of the correlations (indicating only 58% and 43% of the total variance in the right and left eyes respectively) suggests that other factors, in addition to IOP, contribute to the thickness of the RGCs. Factors for consideration include whether IOP elevations were sustained over multiple measurements, IOP level and slope variations, IOP insult-specific vulnerability, and mechanisms of pressure-independent glaucoma (e.g. normal-tension glaucoma) operating in response to glaucoma with elevated pressure.[30] The fact that left eye data had a slightly weaker correlation than right eye data may denote measurement error, or a difference in disease mechanisms whereby one eye is affected more than the other.

4.5 Age and Gender Effects

Thinning of RGCs and associated demographic variables, especially older age in the left eye (r=-0.254, p=0.002) correlating the strongest is consistent with the observed loss of RGCs with advancing age and the population data describing the same phenomenon. Post-mortem studies show a reduction of RGCs by 0.4-0.6% per year in individuals over 40 years of age.[31,32] Age-related and disease-related loss of RGCs makes interpretation of OCT results more challenging and reinforces the necessity of having age-related normative values for the applicable clinical context stemming from the assessment.

CONCLUSION

This study of 144 glaucoma patients identified notable patterns of regional RGC thickness and structure-function correlation in glaucoma. Our primary results indicate that in advanced glaucoma RGC thickness is fairly evenly spread across retinal quadrants, with no notable difference in superiority and inferiority. The pronounced negative correlations between RGC thickness and visual acuity in all quadrants (r=-0.525 to -0.789) and pronounced positive correlations with contrast sensitivity (r=0.539 to 0.769) indicate the value of clinically assessing RGC thickness as a structural marker of visual function.

The pronounced inverse relationship between IOP and RGC thickness further elucidates the destructive role of pressure elevation in the glaucomatous process and the need to prioritize IOP reduction in the management of the disease. The age-related thinning trends of RGCs, left particularly notable in the left eye, illustrate the dual role of aging and the glaucomatous process creating a deficit in RGC populations.

The reported findings are crucial for the revision of glaucoma evaluation protocols. As the positive correlations indicate, a quadrant-wise RGC analysis in its entirety is clinically valuable, in addition to standard visual field testing, for assessing and monitoring glaucoma. The positive correlation with contrast sensitivity suggests that assessing contrast sensitivity may be beneficial in the routine management of glaucoma patients, particularly those who are unable to reliably perform visual field testing.

Further development of longitudinal studies that examine changes in RGC thickness along with functional changes, incorporate detailed visual field analyses, and consider different subtypes and populations of glaucoma, will help refine RGC thickness as a potential clinical biomarker. As the field of OCT (optical coherence tomography) and its OCT analysis algorithms continue to evolve, RGC thickness will be increasingly important to the developed and individualised diagnosis, staging, and management of glaucoma.

In conclusion, this study shows that RGC (retinal ganglion cell) thickness measurements provide a clinically significant structural correlate of function in glaucoma. Regionally and quadrantic analyses provide insights into the disease distribution, and average RGC thickness is a strong functional visual marker. Overall, there is significant evidence to consider the assessment of RGC thickness in adjusting how we manage glaucoma.

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